



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

<b>(51) International Patent Classification:</b> <b>A23L 1/304</b>	<b>A1</b>	<b>(11) International Publication Number:</b> <b>WO 99/23896</b> <b>(43) International Publication Date:</b> 20 May 1999 (20.05.99)
<b>(21) International Application Number:</b> PCT/EP98/06456 <b>(22) International Filing Date:</b> 7 October 1998 (07.10.98) <b>(30) Priority Data:</b> 08/965,665 6 November 1997 (06.11.97) US <b>(71) Applicant (for all designated States except US):</b> SOCIETE DES PRODUITS NESTLE S.A. [CH/CH]; P.O. Box 353, CH-1800 Vevey (CH). <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> JACOBSON, Mark, Randolph [US/US]; 27 Candlewood Springs, New Milford, CT 06776 (US). VADEHRA, Dharam, Vir [US/US]; 6 Halletts Road, New Milford, CT 06776 (US). WEDRAL, Elaine, Regina [US/US]; R.R. 2, P.O. Box 480A, Chestnut Hill Road, Sherman, CT 06784 (US). SHER, Alexander [RU/US]; Apartment 706-S, 5901 Montrose Road, Rockville, MD 20852 (US). MALLANGI, Chandrasekhara, Reddy [US/US]; 2 Briar Lane, New Milford, CT 06776 (US). <b>(74) Agent:</b> PATE, George, Frederick; Avenue Nestlé 55, CH-1800 Vevey (CH).		<b>(81) Designated States:</b> AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GE, GH, GM, HR, HU, ID, IL, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>With international search report.</i> <i>Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.</i>
<b>(54) Title:</b> CALCIUM COMPLEXES FOR FORTIFICATION OF FOODS		
<b>(57) Abstract</b>  A novel calcium complex for the fortification of beverages and foods, especially milk, is disclosed. Fortifying complexes are comprised of a calcium source and a negatively-charged emulsifier with or without an organic or inorganic acid or a salt thereof. These complexes have been found to be particularly effective in fortifying milk and milk-protein containing beverages without coagulation of the proteins or without significantly changing the texture of the product.		

**FOR THE PURPOSES OF INFORMATION ONLY**

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	ES	Spain	LS	Lesotho	SI	Slovenia
AM	Armenia	FI	Finland	LT	Lithuania	SK	Slovakia
AT	Austria	FR	France	LU	Luxembourg	SN	Senegal
AU	Australia	GA	Gabon	LV	Latvia	SZ	Swaziland
AZ	Azerbaijan	GB	United Kingdom	MC	Monaco	TD	Chad
BA	Bosnia and Herzegovina	GE	Georgia	MD	Republic of Moldova	TG	Togo
BB	Barbados	GH	Ghana	MG	Madagascar	TJ	Tajikistan
BE	Belgium	GN	Guinea	MK	The former Yugoslav Republic of Macedonia	TM	Turkmenistan
BF	Burkina Faso	GR	Greece			TR	Turkey
BG	Bulgaria	HU	Hungary	ML	Mali	TT	Trinidad and Tobago
BJ	Benin	IE	Ireland	MN	Mongolia	UA	Ukraine
BR	Brazil	IL	Israel	MR	Mauritania	UG	Uganda
BY	Belarus	IS	Iceland	MW	Malawi	US	United States of America
CA	Canada	IT	Italy	MX	Mexico	UZ	Uzbekistan
CF	Central African Republic	JP	Japan	NE	Niger	VN	Viet Nam
CG	Congo	KE	Kenya	NL	Netherlands	YU	Yugoslavia
CH	Switzerland	KG	Kyrgyzstan	NO	Norway	ZW	Zimbabwe
CI	Côte d'Ivoire	KP	Democratic People's Republic of Korea	NZ	New Zealand		
CM	Cameroon		Republic of Korea	PL	Poland		
CN	China	KR	Republic of Korea	PT	Portugal		
CU	Cuba	KZ	Kazakhstan	RO	Romania		
CZ	Czech Republic	LC	Saint Lucia	RU	Russian Federation		
DE	Germany	LI	Liechtenstein	SD	Sudan		
DK	Denmark	LK	Sri Lanka	SE	Sweden		
EE	Estonia	LR	Liberia	SG	Singapore		

## CALCIUM COMPLEXES FOR FORTIFICATION OF FOODS

### FIELD OF INVENTION

5 The present invention relates to the fortification of foods and beverages, particularly those containing milk proteins, with calcium.

### BACKGROUND OF THE INVENTION

10

Milk is an important source of dietary calcium. Calcium, the most abundant mineral in body, is a major constituent of bone and teeth. This mineral also plays an important role in several physiological systems. Calcium is important  
15 for healthy bone and tooth development in the young and therefore an adequate intake is essential. Calcium status may also be a factor in the development of osteoporosis in elderly people.

20 Since the body does not produce minerals, it is totally dependent on an external supply of calcium, nutritional or supplementary. The importance of adequate calcium intake is recognized during the whole life of the human being. In 1994, the NIH Consensus Development Panel revised  
25 recommended daily allowances for calcium intake for each age group from 800-1200 mg per day to 1500 mg per day.

It has been suggested that calcium in association with caseins may improve absorption in the gastrointestinal  
30 tract. Also it has been found that organic acids salts of calcium are more bioavailable in general than the inorganic salts. Calcium citrate has advantages over other calcium salts for use in fortified foods because of high bioavailability. For example, calcium citrate, as opposed  
35 to calcium in general, has only a marginal effect of interfering with the absorption of other minerals,

especially iron. Also, long-term calcium supplementation with calcium citrate can reduce the risk of formation of kidney and urinary stones since citrate ions are inhibitors for crystallization of stone-forming calcium salts.

5

Addition of calcium to beverages, especially milk, can be very difficult. If slightly or completely insoluble sources of calcium are used, precipitation of the salts can occur especially if stabilizers are not used. If highly soluble sources of calcium (calcium chloride, etc.) are used, interaction between the calcium and calcium sensitive ingredients, such as milk protein, can occur. These interactions can lead to coagulation of the ingredients during temperature treatment even at pasteurization temperature. In addition, the pH of some calcium salt systems may not be compatible with other ingredients or affects the flavor.

10

15

20

25

US patent 4,701,329 and 4,851,243 disclose the use of tribasic calcium phosphate, carrageenan, and guar in calcium- and phosphorous-enriched milk. In this system, the use of stabilizing gums is necessary to prevent sedimentation of the insoluble calcium salt, which also increases the thickness of the milk.

30

US patent 4,840,814 involves a process for preparing calcium enriched milk in which the milk is heat-treated prior to soluble calcium salt addition. This requires additional processing, and could also effect the quality of the milk. In addition, this method is limited to allowing only up to a 30 mg % increase in the calcium.

35

A series of patents such as US 4,722,847, and 4,919,963 (and many subsequent patents), disclose the use of calcium citrate-malate complexes for the fortification of beverages, beverage concentrates, and as supplements. These

systems are stable when the pH is kept below pH 5. For a number of beverages this pH would result in acidic flavors and instability of proteins, especially milk proteins. This problem is also encountered in US patents 4,871,554 and  
5 5,500,232.

EP 0709033 discloses preparation of calcium-supplemented milk drinks through the use of minerals extracted from whey. Although this creates products with good flavor and  
10 stability the level of supplementation is limited to 40mg%.

#### SUMMARY OF THE INVENTION

15 We have developed a complex comprising a calcium source and a negatively-charged emulsifier with or without an organic or inorganic acid or a salt thereof, which may be used to fortify beverages and foods, with improved palatability without affecting product quality.

20 According to the present invention, there is provided a complex formed by the interaction of a suitable calcium source, a negatively charged emulsifier with or without an organic or inorganic acid or a salt thereof.

25 The complexes work particularly well in systems that contain calcium-sensitive components, such as proteins.

#### 30 DETAILED DESCRIPTION OF THE INVENTION

The calcium source that is primarily used to create this complex can include calcium hydroxide, calcium carbonate, calcium chloride, calcium phosphate, calcium sulfate,  
35 calcium nitrate, calcium lactate, calcium fumarate, calcium citrate, calcium acetate, calcium glycerophosphate or

calcium oxide but is preferably calcium hydroxide. The use of an alkaline source, such as calcium hydroxide, advantageously neutralises the pH of the complex. If a non-alkaline calcium source is used, then an alkaline agent  
5 must be added to neutralize the pH of the complex, of which any food grade alkaline agent can be utilized.

The negatively-charged emulsifiers that can be used to form the complex include but are not limited to citric acid  
10 esters of monoglycerides CITREM, (Danisco Ingredients, Inc., New century, KS), stearoyl lactylate (sodium, calcium, or acid), enzyme modified lecithin, stearyl citrate, fatty acids and their salts, or diacetyl tartaric acid esters of monoglycerides. CITREM is most preferred.  
15 The emulsifiers used are not limited to those of a single acyl or fatty acid component, such as on a specific carbon chain length or degree of unsaturation.

The emulsifier used is preferably hydrated, making the  
20 emulsifier more dispersable, and allowing easier exchange with cations. This can be accomplished by various means dependent on the type of emulsifier used, and are commonly known to those familiar with the art. For example, a common method of hydration is by heating a slurry of emulsifier  
25 and water to above 70°C for a period of time (generally more than 10 min.). Once hydrated, the emulsifier dispersion is cooled to near room temperature.

Any one of a number of acids can be used such as citric,  
30 lactic, malic, fumarate, gluconic, succinic, tartaric, or ascorbic, or inorganic acids such as phosphoric. Salts of these acids that can be utilized include potassium, sodium, or calcium salts of the aforementioned acids. For this invention, the most preferred acid is citric acid.

35

Optionally, the complex may be dried and, if desired, stored before further use for fortification of a foodstuff. The amounts needed to form the complex are not critical provided that sufficient amounts of each component are present. Simple mixing of the components is sufficient to form the complex. When an acid is not used in the complex, the weight ratio of calcium (from the calcium source) to surfactant should range from 1:10 to 10:1, and preferably from 2:1 to 1:2. When an acid is used in the complex, the weight ratio of acid to calcium should range from 5:1 to 1:5, and preferably from 2:1 to 1:2, while the weight ratio of calcium to surfactant can range from 100:1 to 1:5. The components are preferably dissolved in water to facilitate mixing and complex formation. The concentration of the solutions is preferably 1-5% by weight or greater. The person of ordinary skill in the art can readily determine convenient amounts to use for any particular application

The complex may conveniently be formed by the interaction of a suitable calcium source, a negatively charged emulsifier, with or without an organic or inorganic acid or a salt thereof. For example, the complex may be prepared by adding acid or a salt of an acid to the emulsifier with mixing, and then adding the calcium source.

When an alkaline calcium source such as calcium hydroxide is added, the pH of the system is neutralized. Alternately, a non-alkaline calcium source can be added, followed by the neutralization with alkaline agent. Any food grade alkaline agent may be used for neutralisation including but not limited to sodium hydroxide, potassium hydroxide, magnesium hydroxide, sodium carbonate, sodium bicarbonate, potassium carbonate and potassium bicarbonate.

The present invention also provides a fortified foodstuff with a fortifying amount of a complex comprising calcium

and a negatively-charged emulsifier with or without an organic or inorganic acid or a salt thereof.

5 The foodstuff may be a dairy based product such as a milk beverage, a liquid nutritional product or other beverage such as a juice, or a confectionary product such as ice cream.

10 The fortified foodstuff comprising a fortifying amount of a complex may be prepared by forming a complex and adding the complex to the foodstuff. The foodstuff can then be heat treated by normal means without any loss in quality such as precipitation, coagulation, or fouling of processing  
15 equipment. The resulting fortified foodstuff is similar to its unfortified counterpart in organoleptic quality. It has a similar colour and taste, without major changes to the texture, viscosity or mouthfeel of the foodstuff.

20 The amount of complex to add to the foodstuff is not critical and is dependent upon the calcium content of the complex and the desired level of fortification. Typically, enough of the complex would be added to fortify the foodstuff from 5% to 200% of the recommended daily allowance for calcium, although even greater amounts are  
25 possible, if desired.

Advantageously, a stabiliser may be added to the foodstuff, preferably before the complex is added to the foodstuff. The stabiliser may be added to the foodstuff in the form of an aqueous solution or suspension or as a dry powder.  
30 Stabilisers that may be used may include but are not limited to carrageenan, xanthan, gellan, pectin, alginates, gumarabic, carboxymethylcellulose, modified and unmodified starches, propylene glycol alginate, locust bean gum, guar gum, hydroxylpropyl cellulose, hydroxypropylmethyl  
35 cellulose, methyl cellulose or mixtures of two or more

thereof. Preferably for dairy products, carrageenan is used as a stabiliser.

5 The following Examples further illustrate the present invention.

#### EXAMPLES

##### 10 Example 1

The following example is intended to demonstrate that a variety of emulsifiers and/or calcium sources can be effective in preparing these complexes.

15 Complexes for fortification of milk were prepared according to the following formulations:

Sample	Emulsifier	Amount	Acid	Amount	Calcium Source	Amount
1	SSL	2.52 g	Citric	1.19 g	Calcium hydroxide	0.84 g
2	CSL	5.25 g	Citric	1.58 g	Does not apply	
3	SSL	2.52 g	Citric	1.19 g	Calcium lactate	3.47 g
4	SSL	3.02 g	Citric	1.41 g	Calcium hydroxide	0.84 g
5	CITREM	2.97 g	Citric	1.19 g	Calcium hydroxide	0.84 g
6	CITREM	2.97	Citric	1.19	Calcium hydroxide	0.90
SSL= Sodium stearyl-2-lactylate, CSL=calcium stearyl-2-lactylate						

Emulsifier was mixed with 50 ml water, the dispersion was heated to 150°F, and then cooled to 100°F. For samples 1-3, a solution of the acid in 10 ml water was then added to the hydrated emulsifier with vigorous mixing. For samples 4 and 5, the acid was added directly to the hydrated emulsifier with vigorous stirring. When applicable, a dispersion of the calcium source in 10 ml water was then added to the emulsifier-acid suspension with vigorous stirring. 6.6g non-fat dry milk was added to 700mL of skim milk under agitation. The resulting complex was added with vigorous stirring to sufficient skim milk (at 120°F) to bring the final volume of calcium fortified milk to 750mL. The pH of the milk was adjusted to 7.0 using a 30% sodium hydroxide needed. The milk was then homogenized at a total pressure of 2500/500 psi using a two-stage APV Rannie® Homogenizer. The fortified milk was filled into 125 ml baby food jars,

pasteurized at 163°F for 15 sec, cooled rapidly in a ice water bath, then stored in a refrigerator.

5 Samples were evaluated for sedimentation and taste after 1 week and for sedimentation after 2 weeks.

#### Results:

10	Sam- ple	0 weeks	1 week	2 weeks
	1	no sed	Sl.sed, sl.Emul flavor	some sed
	2	no sed	Sl.sed, gd flavor	Sl.sed
	3	no sed	Sl.sed, sl.Emul* flavor	some sed
15	4	no sed	no sed, gd flavor	some sed, gd flavor
	5	no sed	no sed, gd flavor	no sed, gd flavor
	6	no sed	no sed, gd flavor	no sed, gd flavor

#### Example 2

20

i) A slurry of 450 g CITREM® was mixed with 15 kg water at room temperature for 60 min. The suspension was heated to 165°F and held at that temperature for 10 min with agitation, then cooled to 90°F (suspension #1). A solution of 544.5 g citric acid in 6.1 kg water was prepared by mixing at room temperature (solution #2). A calcium hydroxide suspension was prepared by mixing 333.0 g calcium hydroxide in 5,000 g water at room temperature (suspension #3). Solution #2 was added to suspension #1 and mix well for 60 min. Suspension #3 was then added and the resulting suspension was mixed for 60 min.

25

30

35

ii) To 10kg skim milk at 40-60°F, 45.0 g carrageenan (SeaKem CM611, FMC Corporation, Philadelphia, PA) was added with mixing for 5 min. The milk was then heated to 165°F and held at 165°F for 5 min under agitation.

iii) To 260kg g skim milk, 2505.0g non-fat dry milk (NFDM) was added at 40-60°F and the milk was mixed for 10 min. The milk was then heated to 120°F, and the carrageenan/milk mixture (ii) was added slowly and the resulting milk was mixed for 5 min. The milk was heated to 149°F and held for 5 min. The milk was cooled to 90°F and the calcium complex was added slowly. The resulting calcium fortified milk was mixed for 10 min and 22.5 g cream/milk flavor was added. The pH of the milk was adjusted with 10% potassium hydroxide solution to 6.9-7.0. The solids content was checked.

iv) Samples were then heat treated under the following conditions.

15

**Pasteurization:**

The calcium fortified milk was homogenized at 120°F and pressure 25000/500psi. The milk was then pasteurized at 163°F for 15 sec. and filled into 330mL glass bottles. The bottles of milk were then cooled in cold water and stored under refrigeration at 40°F.

20

**UHT-pasteurization, plate heat exchanger (PHE):**

Calcium fortified milk was pre-heated to 160°F, then heated to 285°F and held at 285°F for 5 sec, and cooled to 160°F. The milk was then homogenized at pressure 2500/500 psi., cooled to 60°F and filled in 250mL Tetra Brik Aseptic packages (Tetra Pak Inc., Chicago).

25

**UHT-pasteurization steam injection (SI):**

Calcium fortified milk was pre-heated to 175°F, then heated to 285°F by steam injection, held at 285°F for 5 sec, and cooled to 175°F. The milk was homogenised at pressure 2500/500 psi, cooled to 60°F and filled in 250mL Tetra Brik Aseptic packages (Tetra Pak Inc., Chicago).

30

35

**UHT-sterilization (PHE):**

5 Calcium fortified milk was pre-heated to 160°F, then heated to 298°F by plate heat exchangers and held at 298°F for 5 sec, and cooled to 160°F. The milk was homogenised at pressure 2500/500 psi, cooled to 60°F and filled in 250mL Tetra Brik Aseptic packages (Tetra Pak Inc., Chicago).

**UHT-sterilization (SI):**

10 Calcium fortified milk was pre-heated to 175°F, then heated to 298°F by steam injection, held at 298°F for 5sec, and cooled to 175°F. The milk was homogenised at pressure 2500/500 psi, cooled to 60°F and filled in 250mL Tetra Brik Aseptic packages (Tetra Pak Inc., Chicago).

15

The results are given in the following table:

### Results from Example 2

No flocc : No flocculation  
 No ppt : No precipitation  
 slight gel : slight gelation

DESCRIPTION	AGE wk.	STORAGE TEMP (*F)	CENTRIFUGE SEDIMENT (%)	CALCIUM LEVEL** (ppm)	BOILING TEST***	OBSERVATION	SENSORY EVALUATION
Calcium hydroxide citric acid, CITREM Pasteurised	0	40	N/A	1868	No flocc.	No ppt	Good flavour
	2	40	N/A	1854	No flocc.	No ppt	Good flavour
Calcium hydroxide, citric acid, CITREM UHT pasteurized (SI)	0	40	0.03	1890	No flocc.	No ppt	Good flavour
	2	40	0.03	1860	No flocc.	No ppt	Good flavour
	4	40	N/A	1860	No flocc.	No ppt	Good flavour
	6	40	N/A	1860	No flocc.	No ppt	Good flavour
	8	40	N/A	1840	No flocc.	No ppt	Good flavour
Calcium hydroxide, citric acid, CITREM UHT pasteurized (PHE)	0	40	N/A	1894	No flocc.	No ppt	Good flavour
	2	40	N/A	N/A	No flocc.	No ppt	Good flavour
	4	40	N/A	N/A	No flocc.	No ppt	Good flavour
	6	40	N/A	N/A	No flocc.	No ppt	Good flavour
	8	40	N/A	1928	No flocc.	No ppt	Good flavour

Calcium hydroxide, citric acid, CITREM UHT sterilized (SI)	0	80	0.12	1910	No flocc.	No ppt	Good flavour
	2	80	N/A	1860	No flocc.	No ppt	Good flavour
	4	80	N/A	1860	No flocc.	No ppt	Good flavour
	6	80	N/A	1840	No flocc.	No ppt	Good flavour
	8	80	N/A	1850	No flocc.	No ppt	Good flavour
	11	80	0.08	N/A	No flocc.	No ppt	Good flavour
	13	80	0.02	N/A	No flocc.	No ppt	Good flavour
	16	80	0.04	1906	No flocc.	No ppt	Acceptable flavour
						slight gel.	
Calcium hydroxide, citric acid, CITREM UHT sterilized (PHE)	0	80	N/A	1910	No flocc.	No ppt	Good flavour
	2	80	N/A	1860	No flocc.	No ppt	Good flavour
	4	80	N/A	1860	No flocc.	No ppt	Good flavour
	6	80	N/A	1840	No flocc.	No ppt	Good flavour
	8	80	N/A	1850	No flocc.	No ppt	Good flavour
	11	80	N/A	N/A	No flocc.	No ppt	Good flavour
	13	80	N/A	N/A	No flocc.	No ppt	Good flavour
	16	80	N/A	1906	No flocc.	No ppt	Acceptable flavour
						slight gel.	

\* % sediment was determined from sediment weight after centrifugation at 1800g for 5 min and drying of resulting pellet at room temperature overnight

\*\* Total calcium content was determined using a Leeman Labs, model PS 1 AES-ICP spectrometer after dry ashing and as dissolving in nitric acid an water (1:1)

\*\*\* Flocculation was determined by visual inspection. Milk was boiled 15 sec then placed immediately on the convexed surface of a watch glass for inspection

## Example 3

To 25 kg water at 185°F, 720 g CITREM were added and mixed for 5 min., then cooled to 110°F. While under agitation 871 g citric acid were added, and the suspension was mixed for 5 min. 533 g calcium hydroxide was added under agitation and the complex was mixed for 60 min. Steps ii, iii and iv from Example 2 were followed.

The results are given in the following Table :

Descri- ption	Age wk	Storage Temp (°F)	Centrifuge Sediment	Calcium Level** ppm	Boiling test	Observ.	Sensory Eval.
Calcium hydroxide	0	40	0.07	2041	No floc.	No ppt	Gd flav.
citric acid,	2	40	0.04	N/A	No floc.	No ppt	Gd flav.
CITREM, UHT	4	40	0.04	N/A	No floc.	No ppt	Gd flav.
pasteu- rized	6	40	0.04	N/A	No floc.	No ppt	Gd flav.
(SI)	8	40	0.02	2048	No floc.	No ppt	Gd flav.
	10	40	0.04	N/A	No floc.	No ppt	Gd flav.

gd flav. : good flavour

\* % sediment was determined from sediment weight after centrifugation at 1800g for 5 min and drying of resulting pellet at room temperature overnight

\*\* Total calcium content was determined using a Leeman Labs, model PS 1 AES-ICP spectrometer after dry ashing and as dissolving in nitric acid and water (1:1)

\*\*\* Flocculation was determined by visual inspection. Milk was boiled 15 sec then placed immediately on the convexed surface of a watch glass for inspection

Other samples from Example 3 performed very similarly to samples with corresponding heat treatments from Example 2.

#### Example 4

5

This Example shows how the complex can be formed directly in milk.

10

Step 1 from Example 2 was followed to prepare a calcium complex. To 275 kg milk at 40-60°F, 45.0g SeaKem CM 611 carrageenan was added with mixing. To the milk was added 2505.0 g NFDM, 22.5 g cream/milk flavor, the Ca-complex and the resulting fortified milk was mixed for 5 min. The pH was adjusted with 10% potassium hydroxide solution to 6.9-7.0. The solids content was checked. Step iv from example No 2 was then followed.

15

Samples from Example 4 performed very similarly to samples with corresponding heat treatments from Example 2.

20

#### Example 5

25

To 800g water at 185°F, 24.0g CITREM was added and mixed for 5 min, then cooled to <110°F. While under agitation, 29.0g citric acid was added and mixed for 5min. Calcium hydroxide (17.77g) was added under agitation and resulting complex was mixed for 60 min.

30

To 300g skim milk at 40-60°F, 1.5g SeaKem CM 611 carrageenan was added and the milk mixed for 5min. The milk was then heated to 165°F and held at 165°F for 5 min under agitation.

35

To 8.9kg skim milk at 40-60°F, 83.5g NFDM was added and the milk was mixed for 10 minutes. Milk was heated to 120°F and the carrageenan/milk was added slowly and mixed for 5

minutes. Milk was heated to 149°F, held at that temperature for 5 min, then cooled to 90°F. The calcium complex was added slowly, the milk was mixed for 10 min, and 0.75g cream/milk flavor was added. The pH was adjusted with 10% potassium hydroxide solution to 6.9-7.0. The solids content was checked.

---

The calcium fortified milk was placed in 330ml glass jars, autoclaved for 5min at 250°F then cooled to room temperature.

The autoclaved milk fortified with Ca-CITREM-citric acid complex at a total calcium level of 2160ppm performed similarly to the UHT sterilized samples - see Example 2, UHT sterilization (SI).

We Claim:

1. A complex formed by the interaction of a calcium source,  
and a negatively charged emulsifier with or without an  
5 organic or inorganic acid or a salt thereof.
2. A complex according to claim 1 wherein the calcium  
source is calcium hydroxide, calcium carbonate, calcium  
chloride, calcium phosphate, calcium sulfate, calcium  
10 nitrate, calcium lactate, calcium fumarate, calcium  
citrate, calcium acetate, calcium glycerophosphate or  
calcium oxide.
3. A complex according to claim 1 wherein the negatively-  
15 charged emulsifier is a citric acid ester of  
monoglycerides, stearyl lactylate (sodium, calcium, or  
acid), enzyme modified lecithin, stearyl citrate, fatty  
acids and their salts, or a diacetyl tartaric acid esters  
of monoglycerides.  
20
4. A complex according to claim 1 the emulsifier is  
hydrated.
5. A complex according to claim 1 wherein the acid is  
25 citric, lactic, malic, fumaric, gluconic, succinic,  
tartaric, ascorbic, or phosphoric or a salt thereof.
6. A complex according to claim 1 which is dried.
- 30 7. A process of preparing a complex claimed in claim 1  
which comprises interacting a suitable calcium source, a  
negatively charged emulsifier, with or without an organic  
or inorganic acid or a salt thereof.

8. A process of preparing a complex according to claim 7 which comprises adding acid or a salt of the acid to the emulsifier with mixing, and then adding the calcium source.

5 9. A process according to claim 7 wherein, when an alkaline calcium source is added, the pH of the system becomes neutralized.

10 10. A process according to claim 7 wherein when a non-alkaline calcium source is added, this is followed by neutralization with an alkaline agent.

15 11. A fortified foodstuff with a fortifying amount of a complex comprising calcium and a negatively-charged emulsifier with or without an organic or inorganic acid or a salt thereof.

20 12. A fortified foodstuff according to claim 11 wherein the foodstuff is a dairy based product, a liquid nutritional product, a beverage, or a confectionary product.

13. A fortified foodstuff according to claim 11 wherein the foodstuff is a milk beverage, a juice or ice cream.

25 14. A process of preparing a fortified foodstuff claimed in claim 11 which comprises forming a complex and adding the complex to the foodstuff.

30 15. A process of preparing a fortified foodstuff according to claim 14 wherein a stabiliser is added to the foodstuff.

35 16. A process according to claim 15 wherein the stabiliser is carrageenan, xanthan, gellan, pectin, alginates, gum arabic, carboxymethylcellulose, modified and unmodified starches, propylene glycol alginate, locust bean gum, guar gum, hydroxylpropyl cellulose, hydroxypropylmethyl

**SUBSTITUTE SHEET ( rule 26 )**

cellulose, methyl cellulose or mixtures of two or more thereof.

5 17. A fortified foodstuff according to claim 11 wherein the complex is present in an amount sufficient to provide from 5% to 200% of the recommended daily allowance for calcium in the foodstuff.

10 18. A process of preparing a fortified foodstuff according to claim 14 wherein the complex is added in an amount sufficient to provide from 5% to 200% of the recommended daily allowance for calcium in the foodstuff.

**A. CLASSIFICATION OF SUBJECT MATTER**  
IPC 6 A23L1/304

According to International Patent Classification (IPC) or to both national classification and IPC

**B. FIELDS SEARCHED**Minimum documentation searched (classification system followed by classification symbols)  
IPC 6 A23L

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

**C. DOCUMENTS CONSIDERED TO BE RELEVANT**

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5 063 074 A (KAHN MARVIN L ET AL) 5 November 1991 see claims 1-7; example 1 ---	1-3,7, 11-18
X	US 5 514 387 A (ZIMMERMAN ELLEN L ET AL) 7 May 1996 see claims 1-36; examples 1-4 ---	1-3,7, 11,12,17
X	EP 0 614 612 A (GEN FOODS INC) 14 September 1994 see example 15 --- -/--	1-3,7,11

☒ Further documents are listed in the continuation of box C.☒ Patent family members are listed in annex.

## \* Special categories of cited documents :

- "A" document defining the general state of the art which is not considered to be of particular relevance
- "E" earlier document but published on or after the international filing date
- "L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- "O" document referring to an oral disclosure, use, exhibition or other means
- "P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search

12 April 1999

Date of mailing of the international search report

29/04/1999

Name and mailing address of the ISA

European Patent Office, P.B. 5818 Patentlaan 2  
NL - 2280 HV Rijswijk  
Tel. (+31-70) 340-2040, Tx. 31 651 epo nl,  
Fax: (+31-70) 340-3016

Authorized officer

De Jong, E

## C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	<p>DATABASE WPI  Section Ch, Week 9717  Derwent Publications Ltd., London, GB;  Class D13, AN 97-186973  XP002099471  &amp; JP 09 047269 A (NIPPON DEL MONTE KK)  , 18 February 1997  see abstract</p> <p>---</p>	1-18
A	<p>DATABASE WPI  Section Ch, Week 7839  Derwent Publications Ltd., London, GB;  Class D13, AN 78-69623A  XP002099472  &amp; JP 53 096356 A (IWASHITA N)  , 23 August 1978  see abstract</p> <p>---</p>	1-18
A	<p>PATENT ABSTRACTS OF JAPAN  vol. 095, no. 008, 29 September 1995  &amp; JP 07 138018 A (MARUO CALCIUM CO LTD),  30 May 1995  see abstract</p> <p>---</p>	1-18
A	<p>HIROTSUKA M ET AL: "Calcium fortification  of soy milk with calcium-lecithin liposome  system."  JOURNAL OF FOOD SCIENCE,  vol. 49, no. 4, 1984, pages 1111-1112,  1127, XP002099470  Res. Inst. for Food Sci., Kyoto Univ.,  Uji-Kyoto 611, Japan  see the whole document</p> <p>---</p>	1-18
A	<p>WO 94 00107 A (PROCTER &amp; GAMBLE)  6 January 1994  see page 11</p> <p>---</p>	1-18
A	<p>US 4 919 963 A (HECKERT DAVID C)  24 April 1990  cited in the application  see claims 1-11</p> <p>-----</p>	1-18

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
US 5063074 A	05-11-1991	US 5175015 A	29-12-1992
US 5514387 A	07-05-1996	NONE	
EP 0614612 A	14-09-1994	US 5258190 A	02-11-1993
		CA 2114997 A	11-09-1994
		CZ 9400300 A	15-12-1994
		HU 69632 A	28-09-1995
		PL 174303 B	31-07-1998
		SK 24394 A	05-01-1995
WO 9400107 A	06-01-1994	AU 4597293 A	24-01-1994
		MX 9303922 A	29-04-1994
US 4919963 A	24-04-1990	US 4722847 A	02-02-1988
		AT 55042 T	15-08-1990
		AU 594271 B	01-03-1990
		AU 7253387 A	12-11-1987
		CA 1325130 A	14-12-1993
		EG 18049 A	30-08-1991
		EP 0244903 A	11-11-1987
		FI 872007 A,B,	08-11-1987
		GR 3000729 T	10-10-1991
		IE 60333 B	29-06-1994
		JP 2559732 B	04-12-1996
		JP 63052864 A	07-03-1988
		KR 9604263 B	30-03-1996
		MX 165456 B	11-11-1992
		PH 23972 A	23-01-1990
		PH 27164 A	02-04-1993
		PT 84820 A,B	01-06-1987
		TR 24771 A	01-05-1992



CHWANG, T., Ling  
Hitt Chwang & Gaines, P.C.  
Suite 225  
275 West Campbell Road  
Richardson, TX 75080

ETATS-UNIS D'AMERIQUE

Datum/Date

04/09/00

Zeichen/Ref./Réf.

Anmeldung Nr./Application No./Demande n°/Patent Nr./Patent No./Brevet n°.

00915784.3- -PCT/US0003993

Anmelder/Applicant/Demandeur/Patentinhaber/Proprietor/Titulaire

MORNINGSTAR DIAGNOSTICS, INC.

#### ENTRY INTO THE EUROPEAN PHASE BEFORE THE EUROPEAN PATENT OFFICE

NOTE: These notes describes the procedural steps required for entry into the European phase before the European Patent Office (EPO). You are advised to read them carefully; failure to take the necessary action in time can lead to your application being deemed withdrawn.

1. European patent application no. 00915784.3 has been allotted to the above-mentioned international patent application.
2. Applicants WITHOUT a residence or their principal place of of business within the territory of an EPC Contracting State may themselves initiate European processing of their international application, provided they do so before expiry of the 21st or 31st month from the the priority date (see also point 7 below).

During the European phase before the EPO as designated or elected Office, however, such applicants must be represented by a professional representative (Articles 133(2) and 134(7) EPC).

Procedural acts performed after expiry of the 21st or 31st month by a professional representative who acted during the international phase but is not authorised to act before the EPO have no legal effect and therefore lead to loss of rights.

Please note that a professional representative authorised to act before the EPO and who acted for the applicant during the international phase does not automatically become the representative for the European phase. Applicants therefore strongly advised to appoint in good time any representative they wish to initiate the European phase for them; otherwise, the EPO has to send all communications direct to the applicant.





3. Applicants WITH a residence or their principal place of business within the territory of an EPC Contractin State are not obliged to appoint a professional representative authorised to act before the EPO for the European phase before the EPO as a designated or elected Office.  
However, in view of the complexity of the procedure it is recommend d that they do so.
4. Applicants and professional representatives are strongly advised to initiate the European phase using EPO Form 1200 (available free of charge from the EPO). This however is not compulsory.
5. TO ENTER THE EUROPEAN PHASE BEFORE THE EPO, the following acts must be performed. (NB: Failure validly to do so will entail loss of rights or other adverse legal consequences).
  - 5.1 If the EPO acting as DESIGNATED OFFICE under Article 22(1) PCT, applicants must, within 21 months from the date of filing or (where applicable) the earliest priority date:
    - a) Supply a translation of the international application into an EPO official language, if the International Bureau did not publish the application in such a language (Article 22(1) PCT and Rule 107(1)a) EPC).  
If the translation is not filed in due time, the international application is deemed to be withdrawn before the EPO (Article 24(1)(iii) PCT).
    - b) Pay the national basic fee and, where a supplementary European search report has to be drawn up, the search fee (Rule 107(1)c) and e) EPC).
    - c) Within six months from publication of the international search report, pay a designation fee for each designated Contracting State (Rule 107(1)d) EPC), and file a written request for examination and pay the examination fee (Rule 107(1)f) EPC).

Anmeldung Nr./Application No./Demande n°./Patent Nr./Patent No./Brevet n°.	Blatt/Page/Feuille
00915784.3	2





- 5.2 If the EPO is acting as ELECTED OFFICE under Article 39(1)a) PCT, applicants must, within 31 months from the date of filing or (where applicable) the earliest priority date:
- a) File a translation as per 5.1 a) above.
  - b) Pay the fees as per 5.1 b) above.
  - c) If the time limit under Article 79(2) EPC expires before the 31-month time limit, pay the designation fee for each designated Contracting State (Rule 107(1)d) EPC).
  - d) If the time limit under Article 94(2) EPC expires before the 31-month time limit, file the written request for examination A N D pay the examination fee (Rule 107(1)f) EPC).
  - e) Pay the renewal fee for the third year, if it falls due before the expiry of the 21-month time limit (Rule 107(1)g) EPC)
- 5.3 If the application documents on which the European grant procedure is to be based comprise more than ten claims, a claims fee is payable within the time limit under Rule 107(1) EPC for the eleventh and each subsequent claim (Rule 110(1) EPC). The fee can however still be paid within a period of grace of one month from notification of an EPO communication (Rule 110(2) EPC).
6. If the necessary fees are not paid in time, they may still be validly paid within a period of grace of one month from notification of an EPO communication, subject to payment at the same time of a surcharge for each late-paid fee (Rule 85a(1), 85b EPC). For the renewal fee, the period of grace is six months from the fee's due date (Article 86(2) EPC).
7. If the applicant had a representative during the application's international phase, the present notes will be sent to the representative, asking him to inform the applicant accordingly.

All subsequent communications will be sent to the applicant, or - if the EPO is informed of his appointment in time - to the applicants' European representative.





8. For more details about time limits and procedural acts before the EPO as designated and elected Office, see the EPO brochure

How to get a European patent  
Guide for applicants - Part 2  
PCT procedure before the EPO - "EURO-PCT"

This brochure, the list of professional representatives before the EPO, Form 1200 and the latest fees are all on the internet under

<http://www.european-patent-office.org>.

RECEIVING SECTION



Anmeldung Nr./Application No./Demande n°./Patent Nr./Patent No./Brevet n°. 00915784.3	Blatt/Page/Feuille 4
--	-------------------------

